IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: J. Clark & C. Denning	Art Unit: 1632
Filing Date: June 13, 2000	Examiner: Qian J. Li, Ph.D.
Serial No: 09/593,316	
Docket: 730/002	
Title: ANIMAL TISSUE FOR XENOTRANSPLANTATION	

DECLARATION UNDER 37 CFR § 1.132
BY IAN WILMUT, Ph.D., O.B.E., F.R.S.

Commissioner for Patents
Alexandria VA 22313

Dear Sir:

I, IAN WILMUT, do hereby declare as follows:

I am head of the Department of Gene Function and Development at the Roslin Institute in Midlothian, Scotland. The group headed by Keith Campbell and myself cloned Dolly the Sheep — the first mammal to be cloned from an adult cell. The methods we used are described in U.S. Patent Nos. 6,147,276; 6,252,133; and 6,525,243.

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I have reviewed the Patent Application by John Clark and Chris Denning referred to at the top of this Declaration. I understand the Examiner has questioned whether genetically qualified animals can be made according to the Campbell and Wilmut method, and whether a homozygous α(1,3)galactosyltransferase knockout sheep can be made using the gene sequence information provided in this patent application.

After the sheep α1,3GT gene was isolated as described in the application, Dr. Clark and Dr. Denning turned their attention to making α1,3GT knockout sheep. Limited resources were available to pursue the project, and the project was not completed. Preliminary results were reported in the research article published by C. Denning et al., Nature Biotech. 19:559, 2004, for which I am a coauthor.

The paper reports that heterozygous α1,3GT knockout cells were produced as donor cells for cloning by nuclear transfer, but no fetus survived the full term of pregnancy. When the longest lived fetus was autopsied, we found abnormalities around the blood vessels in the lung, which were apparently fatal.

Accompanying this Declaration is an article which I coauthored with Susan Rhind et al. (Nature Biotech. 21:744, 2003). The article explains that lung abnormalities of this kind are seen in failed neonatal cloned sheep more often than they are seen in normal sheep pregnancies. Amongst the animals listed in Table 1, animals 1, 3, 4, and 5 were cloned from knockout cells; animal 6 was cloned from a cell containing a randomly integrated transgene, and animals 2, 7, and 8 were cloned from cells without any genetic modification. Lung abnormalities were seen in cases 1, 3, 5, 6, and 7, which means that the abnormality is not attributable to the use of genetically modified cells, but is an artifact of the cloning process in general. We believe the lung abnormalities are due to incomplete reprogramming when the nucleus of the donor cell is transferred to the recipient oocyte during cloning.

The Denning article shows that there was a frequency of failed pregnancies whether they contained a α1,3GT knockout (Table 2, lines 3C6 and 3E1), a randomly integrated gene (4H2), a knockout of the PrP gene (YH6), or were cloned from unaltered cells (7G65F4). Failure of the α1,3GT knockouts was not attributable to the genetic modification, which has no known relationship to the viability of smooth muscle cells. Rather, it reflects the rate of failure in this series of experiments, irrespective of what genetic modifications were made. As explained in the article, we attribute the rate of failure to the number of doublings of the cells in tissue culture.

There is no reason why genetically modified animals cannot be made according to the method that Keith Campbell described in our patent disclosures. Using donor cells that have not undergone extensive culturing, or that have a long replicative capacity may improve the frequency of successful cloning. But it is only the frequency that is affected, not the ultimate efficacy. It is my belief that cultured cell lines such as those used by Denning et al. will successfully generate cloned animals after sufficient persistence.

This is illustrated by the successful cloning of heterozygous and homozygous knockouts and transgenic animals by other laboratories, using the Campbell and Wilmut technique. For example, Phelps et al. (Science 299, 411-414, 2003) and Kolber-Simonds et al. (Proc. Natl. Acad. Sci. USA 101:7335, 2004) have both cloned α1,3GT knockout pigs. Kuroiwa et al. (Nature Genetics 36:775, 2004) cloned cattle that contain homozygous knockouts of both the IgM μ-chain gene, and the PrP gene.

The cloning method used by all these groups is the same as described by Keith Campbell and myself in our U.S. Patents. There is no modification to any aspect of our method — selection of the oocyte, transfer of the nucleus, activation of the combined cell, or implantation into the surrogate female — that is needed for the method to work when the donor cell has been genetically modified.

The patent application by Denning and Clark provides the sheep α1,3GT gene, and describes the making of targeting vectors, and knockout cells. Making α1,3GT knockout sheep from the sheep α1,3GT gene should be no more difficult than making α1,3GT knockout pigs from the pig α1,3GT gene.

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I hereby declare that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Sept 9, 2004
Date

Ian Wilmut, Ph.D.
Midlothian, Scotland